



Organic Chemistry

Third Edition

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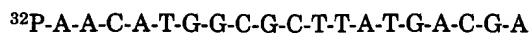
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PROBLEM.....

- 29.21 Show the labeled cleavage products you would expect to obtain if the following DNA segment were subjected to each of the four cleavage reactions:



PROBLEM.....

- 29.22 Sketch what you would expect the gel electrophoresis pattern to look like if the DNA segment in Problem 29.21 were sequenced.

PROBLEM.....

- 29.23 Finish assigning the sequence to the gel electrophoresis pattern shown in Figure 29.17.
-

29.17 Laboratory Synthesis of DNA

The development of genetic engineering techniques in the last two decades brought with it an increased demand for efficient chemical methods for the synthesis of short DNA segments. Ideally, whole genes might be synthesized in the laboratory and inserted into the DNA of microorganisms, thereby directing the microorganisms to produce the specific protein coded for by that gene—perhaps insulin or some other valuable material.

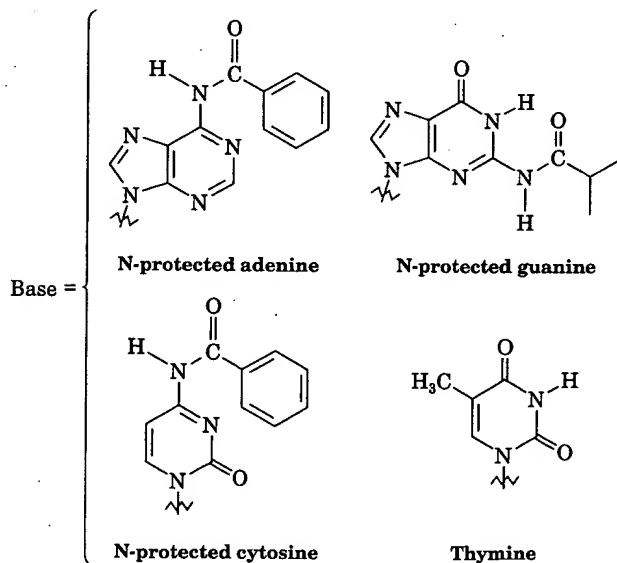
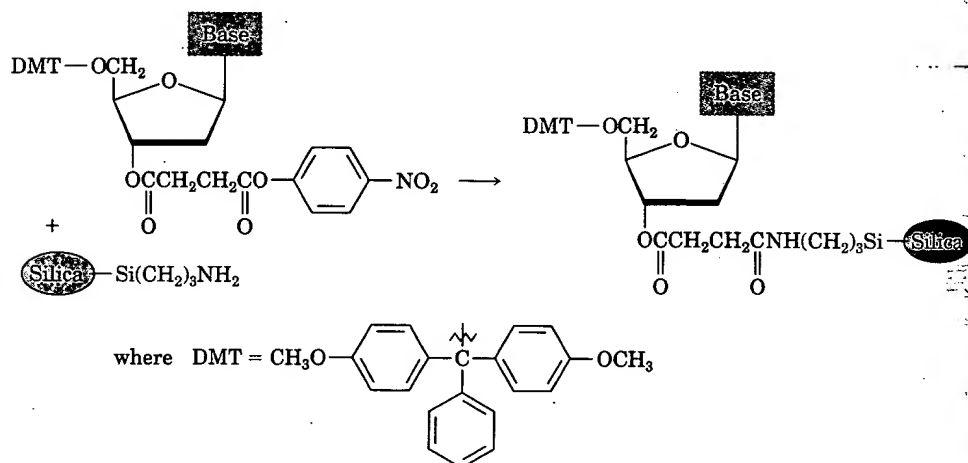
The problems of DNA synthesis are similar to those of protein synthesis (Section 27.11) but are considerably more difficult because of the structural complexity of the deoxyribonucleotide monomers. Each nucleotide has several reactive sites that must be selectively protected and deprotected at the proper times, and coupling of the four nucleotides must be carried out in the proper sequence. Despite these difficulties, some extremely impressive early achievements were recorded, most notably the synthesis by Khorana⁴ in 1979 of the tyrosine suppressor tRNA gene from the bacterium *Escherichia coli*. Some 207 base pairs were assembled in an effort that required 10 years of work.

More recently, automated “gene machines” have become available, which allow the fast and reliable synthesis of DNA sequences 100–200 nucleotides long. These DNA synthesizers operate on a principle similar to that of the Merrifield solid-phase peptide synthesizer (Section 27.12). In essence, a protected nucleotide is covalently bound to a solid support, and one nucleotide at a time is added to the chain. When the last nucleotide has been added, the protecting groups are removed, and the synthetic DNA is cleaved from the solid support.

⁴Har Gobind Khorana (1922–); b. Raipur, India; Ph.D. University of Liverpool; professor, Massachusetts Institute of Technology; Nobel Prize in medicine (1968).

Step 1 in DNA synthesis involves attachment of a protected deoxynucleoside to a silica (SiO_2) support by an ester linkage to the deoxynucleoside's 3' position. Both the 5' hydroxyl and free amino groups on the heterocyclic bases must be protected to accomplish this attachment. Adenine and cytosine bases are protected by benzoyl groups, guanine is protected by an isobutyryl group, and thymine requires no protection. The deoxyribose 5' hydroxyl is protected as its DMT ether, where DMT = *p*-dimethoxytrityl. Other protecting groups can also be used, but those mentioned here are common choices.

Step 1



Step 2 involves deprotection of the 5'-deoxyribose hydroxyl by treatment with dichloroacetic acid in CH_2Cl_2 to remove the DMT group. The reaction proceeds rapidly by an $\text{S}_{\text{N}}1$ mechanism because of the stability of the tertiary, triply benzylic dimethoxytrityl cation.

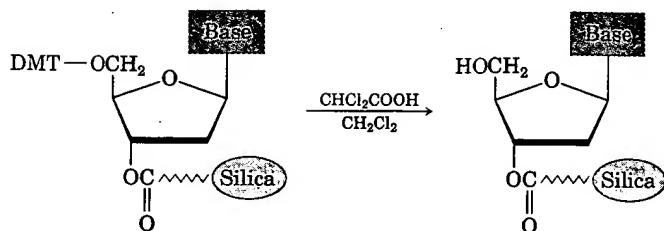
Step 2

Step 3
a protected
position. [A
 $\text{R}_2\text{NP}(\text{OR})_2$
acetonitrile
yields a *ph*
Note that o
group. The

Step 3

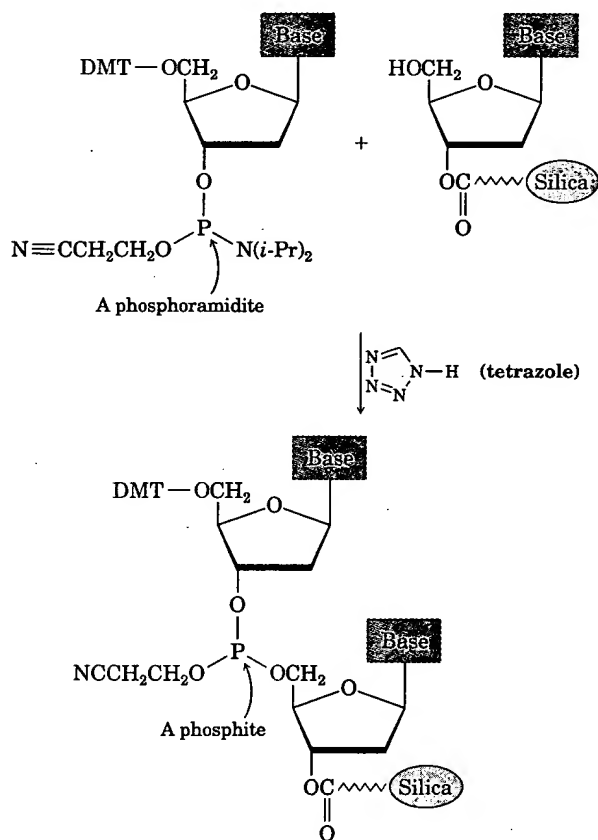
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Step 2



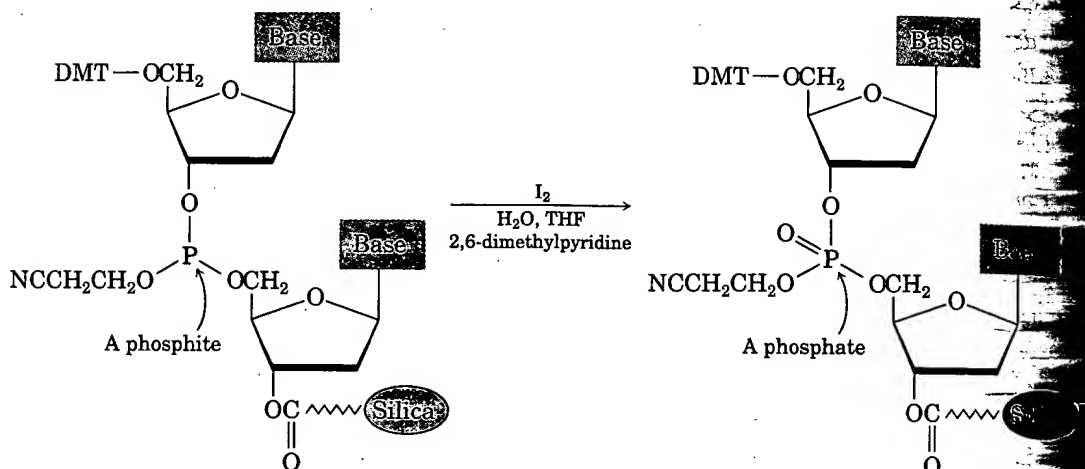
Step 3 involves coupling of the polymer-bonded deoxynucleoside with a protected deoxynucleoside containing a *phosphoramidite* group at its 3' position. [A phosphoramidite has a trivalent phosphorus with the structure $\text{R}_2\text{NP}(\text{OR})_2$.] The coupling reaction takes place in the polar aprotic solvent acetonitrile, requires catalysis by the heterocyclic amine tetrazole, and yields a *phosphite*, or trialkoxyphosphorus compound, $\text{P}(\text{OR})_3$, as product. Note that one of the phosphorus oxygen atoms is protected by a β -cyanoethyl group. The coupling step takes place in better than 99% yield.

Step 3



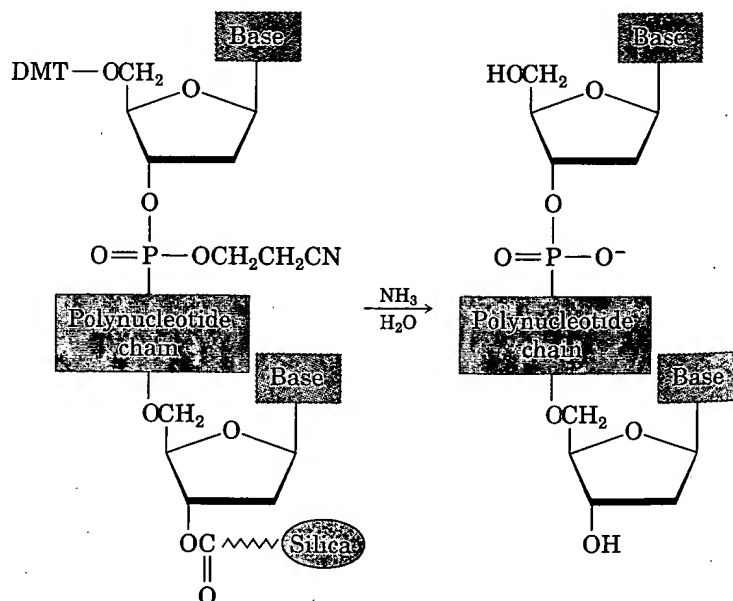
With the coupling completed, the phosphite product is then oxidized to a phosphate triester by treatment with iodine. The reaction is carried out in aqueous tetrahydrofuran in the presence of 2,6-dimethylpyridine.

Step 4



The cycle of steps—deprotection, coupling, and oxidation—is then repeated until a polydeoxyribonucleotide chain of the desired length and sequence has been built. The final step is to cleave all protecting groups from the heterocyclic bases and from the phosphates and to cleave the ester bond holding the DNA to the silica. All of these reactions are done at the same time by treatment with aqueous ammonia. Purification by electrophoresis then yields the synthetic DNA.

Step 5



PROBLEM.....

29.25 Propose a mechanism for the phosphorylation reaction shown.

29.18 Summary

A heterocycle of atom. Nitrogen heterocycles display aromaticity. Aromatic heterocycles are the simplest and most common with electrophilic aromatic substitution next to the nucleobases.

Pyridine is a heterocyclic aromatic amine. It is a weak base and a good nucleophile. It is used in the synthesis of nucleic acids.

The nucleic acid is a polymer of nucleotides. A nucleotide is composed of a phosphate group, a sugar, and a nitrogenous base. The sugar is deoxyribose in DNA and ribose in RNA. The nitrogenous base is one of the four bases: adenine, guanine, cytosine, and thymine (or uracil in RNA).

PROBLEM.....

29.24 *p*-Dimethoxytrityl (DMT) ethers are easily cleaved by mild acid treatment. Show the mechanism, and suggest a reason why this ether cleavage is unusually easy.

The nucleotide is a nucleic acid. A nucleic acid is a polymer of nucleotides. A nucleotide is composed of a phosphate group, a sugar, and a nitrogenous base. The sugar is deoxyribose in DNA and ribose in RNA. The nitrogenous base is one of the four bases: adenine, guanine, cytosine, and thymine (or uracil in RNA).

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